

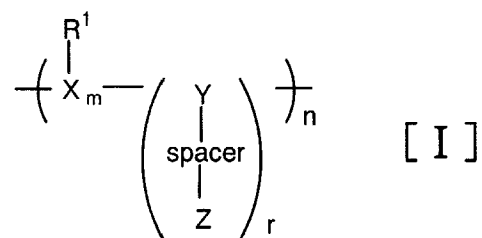
AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Amend the paragraph beginning on page 8, line 26 as follows:

The present invention relates to: a cell culture substrate coated with a hydrophobic binding-absorptive polymer having a hydrophobic linear skeleton and a functional group which can react to a protein or a peptide in a molecule (“1”), the cell culture substrate ~~according to “1”~~, wherein a base material of the cell culture substrate comprises a biobased polymer, plastic, natural or synthetic rubber, an inorganic material or metal (“2”), the cell culture substrate ~~according to “2”~~, wherein the biobased polymer is collagen, gelatin, cellulose, agarose, alginic acid, chitin, chitosan, or a biodegradable polymer, which is, polylactic acid, polybutylente succinate, or polycaprolactone (“3”), the cell culture substrate ~~according to “2”~~, wherein the plastic is a thermoplastic resin or a thermosetting resin (“4”), the cell culture substrate ~~according to “4”~~, wherein the thermoplastic resin is an acryl resin, a polyvinyl chloride resin, a polyethylene resin, a polystyrene resin, a polypropylene resin, a polymethylpentene resin or a fluorocarbon resin (“5”), the cell culture substrate ~~according to “4”~~, wherein the thermosetting resin is a phenolic resin, an urea formaldehyde resin, an epoxy resin, a melamine resin or a silicone resin (“6”), the cell culture substrate ~~according to “2”~~, wherein the synthetic rubber is butadiene-styrene rubber, butadiene-acrylonitrile rubber, butyl rubber, polysulfide-based synthetic rubber, fluorocarbon rubber or silicone rubber (“7”), the cell culture substrate ~~according to “2”~~, wherein the inorganic material is glass, hydroxyapatite, IC substrate or a carbon nanotube (“8”), the cell culture substrate ~~according to “2”~~, wherein the metal is inert

gold, platinum, titanium, indium, or an oxide thereof which is titanium oxide, indium oxide, or ITO (indium tin oxide) (~~“9”~~), the cell culture substrate ~~according to “1”~~, wherein the cell culture substrate comprising a base material ~~according to “2” to “9”~~ is a well, a printed-wiring board, or an artificial organ (~~“10”~~), the cell culture substrate ~~according to “10”~~, wherein the artificial organ is an artificial blood vessel, an artificial heart lung, or an artificial kidney (~~“11”~~), the cell culture substrate ~~according to “1” or “10”~~, wherein the cell culture substrate is a well comprising silicone as a base material (~~“12”~~), the cell culture substrate ~~according to any one of “1” to “12”~~, wherein the hydrophobic binding-adsorptive polymer is shown by the following formula [I]:



(wherein, X denotes CH or NHCHCO, Y denotes CH or NHCR²CO, R¹ denotes H, alkyl group of carbon number 1 to 10, alkoxy group of carbon number 1 to 10, aryl or aralkyl group of carbon number 6 to 10, or aryloxy or aralkyloxy group of carbon number 6 to 10, R² denotes H or alkyl group of carbon number 1 to 10, Z denotes a functional group (reactive group) and is optionally bonded to X reciprocally, spacer denotes (-CH₂)_p or (-NHCHR³CO-)q, R³ denotes H or alkyl group of carbon number 1 to 10, m denotes an integral number greater or equal to 1, n denotes an integral number between 100 and 20000, p and q independently denote 0 or integral numbers 1 to 8, and r denotes an integral number greater or equal to 1) (~~“13”~~), the cell culture

substrate ~~according to "13"~~, wherein the hydrophobic binding-adsorptive polymer shown by the formula [I] is a copolymer made of a vinyl-based compound and maleic anhydride (~~"14"~~), and the cell culture substrate ~~according to "14"~~, wherein the vinyl-based compound is methyl vinyl ether, ethyl vinyl ether, butyl ether, hexyl vinyl ether or styrene (~~"15"~~).

Amend the paragraph beginning on page 11, line 3 as follows:

The present invention also relates to: an immobilized preparation of a cell adhesion protein or peptide wherein the cell adhesion protein or peptide is bound to the cell culture substrate ~~according to any one of "1" to "15" ("16")~~, the immobilized preparation ~~according to "16"~~, wherein the binding is covalent bonding formed by a reaction between a functional group, which is capable of reacting to a protein or a peptide, of a hydrophobic binding-adsorptive polymer and a reactive group of a cell adhesion protein or peptide (~~"17"~~), the immobilized preparation ~~according to "17"~~, wherein the covalent bonding is amide bonding (~~"18"~~), the immobilized preparation ~~according to any one of "16" to "18"~~, wherein the cell adhesion protein is fibronectin (FN), collagen (Col), laminin (LN) or vitronectin (VN) (~~"19"~~), the immobilized preparation ~~according to any one of "16" to "18"~~, wherein the cell adhesion peptide is a peptide in a region relating to cell adhesion in an amino acid sequence of the cell adhesion protein ~~according to "19" ("20")~~, the immobilized preparation ~~according to "20"~~, wherein the peptide in a region relating to cell adhesion of fibronectin (FN) protein is a peptide having a specific Arg-Gly-Asp (RGD) amino acid sequence which binds to an integrin receptor on a cell side (~~"21"~~), the immobilized preparation ~~according to "21"~~, wherein the peptide having an RGD amino acid

sequence is Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser (FIB-1) (SEQ ID NO: 16) ("22"), the immobilized preparation ~~according to "20"~~, wherein the peptide in a region relating to cell adhesion of laminin (LN) protein is an α -chain G-domain peptide ("23"), the immobilized preparation ~~according to "23"~~, wherein the G-domain peptide is Arg-Lys-Arg-Leu-Gln-Val-Gln-Leu-Ser-Ile-Arg-Thr (AG73) (SEQ ID NO: 1), Leu-Gln-Gln-Arg-Arg-Ser-Val-Leu-Arg-Thr-Lys-Ile (AG73T) (SEQ ID NO: 2), Thr-Leu-Gln-Leu-Gln-Glu-Gly-Arg-Leu-His-Phe-Met (AG76.8) (SEQ ID NO: 17), Thr-Leu-Gln-Leu-Gln-Glu-Gly-Arg-Leu-His-Phe-Nle (AG76.8X) (SEQ ID NO: 18), Val-Lys-Thr-Glu-Tyr-Ile-Lys-Arg-Lys-Ala-Phe-Met (AG81.2) (SEQ ID NO: 3), Val-Lys-Thr-Glu-Tyr-Ile-Lys-Arg-Lys-Ala-Phe-Nle (AG81.2X) (SEQ ID NO: 4), Lys-Asn-Arg-Leu-Thr-Ile-Glu-Leu-Glu-Val-Arg-Thr (A2G73) (SEQ ID NO: 5), Lys-Pro-Arg-Leu-Gln-Phe-Ser-Leu-Asp-Ile-Gln-Thr (A3G72) (SEQ ID NO: 6), Lys-Phe-Leu-Glu-Gln-Lys-Ala-Pro-Arg-Asp-Ser-His (A4G73) (SEQ ID NO: 19), Gly-Glu-Lys-Ser-Gln-Phe-Ser-Ile-Arg-Leu-Lys-Thr (A4G78) (SEQ ID NO: 20), Thr-Leu-Phe-Leu-Ala-His-Gly-Arg-Leu-Val-Phe-Met (A4G82) (SEQ ID NO: 7), Thr-Leu-Phe-Leu-Ala-His-Gly-Arg-Leu-Val-Phe-Nle (A4G82X) (SEQ ID NO: 8), Gly-Pro-Leu-Pro-Ser-Tyr-Leu-Gln-Phe-Val-Gly-Ile (A5G71) (SEQ ID NO: 9), Arg-Asn-Arg-Leu-His-Leu-Ser-Met-Leu-Val-Arg-Pro (A5G73) (SEQ ID NO: 10), Arg-Asn-Arg-Leu-His-Leu-Ser-Nle-Leu-Val-Arg-Pro (A5G73X) (SEQ ID NO: 11), Leu-Val-Leu-Phe-Leu-Asn-His-Gly-His-Phe-Val-Ala (A5G77) (SEQ ID NO: 12), Leu-Val-Leu-Phe-Leu-Asn-His-Gly-His (A5G77f) (SEQ ID NO: 13), Lys-Asn-Ser-Phe-Met-Ala-Leu-Tyr-Leu-Ser-Lys-Gly (hA3G75) (SEQ ID NO: 21) or Gly-Asn-Ser-Thr-Ile-Ser-Ile-Arg-Ala-Pro-Val-Tyr (hA3G83) (SEQ ID NO: 15) ("24"), the immobilized preparation ~~according to "20"~~, wherein the cell adhesion peptide is a

peptide comprising 3 to 20 amino acid residues ("25"), a method for producing a immobilized preparation wherein a functional group, which is capable of reacting to a protein or a peptide, of a hydrophobic binding-adsorptive polymer coated on a cell culture substrate reacts to a cell adhesion protein or peptide ("26"), a method for producing a immobilized preparation wherein a functional group, which is capable of reacting to a protein or a peptide, of a hydrophobic binding-adsorptive polymer reacts to a cell adhesion protein or peptide, and a cell culture substrate is coated with the reactant ("27"), and a reactant obtained by reacting a functional group, which is capable of reacting to a protein or a peptide, of a hydrophobic binding-adsorptive polymer, to a cell adhesion protein or peptide ("28").

Amend the paragraph beginning on page 13, line 5 as follows:

The present invention further relates to: an artificial tissue prepared by seeding a desired cell on the immobilized preparation of a cell adhesion protein or peptide ~~according to any one of "16" to "27"~~, and culturing the cell ("29"), the artificial tissue ~~according to "29"~~, wherein the desired cell is an epithelial cell, an endothelial cell or a mesenchymal cell ("30"), the artificial tissue ~~according to "30"~~, wherein the epithelial cell is an epidermal cell, a corneal epithelial cell, an alveolar epithelial cell, a mucosal epithelial cell of digestive system, a renal glomerular epithelial cell or a hepatic parenchymal cell ("31"), the artificial tissue ~~according to "30"~~, wherein the endothelial cell is a renal glomerular ciliated cell, a vascular endothelial cell, a pulmonary arterial vascular endothelial cell, a placental venous vascular endothelial cell or an aortic endothelial cell ("32"), the artificial tissue ~~according to "30"~~, wherein the mesenchymal

cell is a muscle cell, an adipocyte, a glial cell, a Schwann cell or a neural cell (neuron) the artificial tissue ~~according to any one of “29” to “33”~~, wherein the artificial tissue is an artificial epidermal tissue, an artificial corneal epithelial tissue, an artificial alveolar epithelial tissue, an artificial respiratory epithelial tissue, an artificial renal glomerular tissue, an artificial hepatic parenchymal tissue or an artificial vascular endothelial tissue, or an artificial blood vessel, an artificial lung, an artificial liver, an artificial kidney, an artificial skin or an artificial cornea (“34”).

Amend the paragraph beginning on page 16, line 6 as follows:

FIG. 15 is a graph showing that the adhesion level of T2 cells suspended in the serum-free medium changed in a manner depending on the coated amounts of pseudomatrices MAST-GRGDSP (SEQ ID NO: 22) and MMAC-GRGDSP (SEQ ID NO: 22) (0.1-1.0 µg/well) that were diluted to the concentration of 2-20 µg/ml with 50% ethanol, poured into wells by 50 µl each, and air-dried/immobilized, by adopting a case where FN was coated, as a standard.

Amend the paragraph beginning on page 16, line 13 as follows:

FIG. 16 is a graph showing that the adhesion of T2 cells to pseudomatrix MAST-GRGDSP (SEQ ID NO: 22) (0.1-1.0 µg/well) which was diluted with 50% ethanol, poured into wells, and air-dried/immobilized, was competitively inhibited by the free GRGDSP (SEQ ID NO: 22) peptide.

Amend the paragraph beginning on page 16, line 17 as follows:

FIG. 17 is a graph showing that the adhesion of T2 cells to pseudomatrix MMAC-GRGDSP (SEQ ID NO: 22) (0.1-1.0 µg/well) which was diluted with 50% ethanol, poured into wells, and air-dried/immobilized, was competitively inhibited by the free GRGDSP (SEQ ID NO: 22) peptide.

Amend the paragraph beginning on page 25, line 16 as follows:

As a peptide in the region relating to the cell adhesion of FN protein, a peptide having the specific RGD amino acid sequence which binds to an integrin receptor on a cell side is preferable, and as a specific sequence, Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser (FIB-1) (SEQ ID NO: 16) is exemplified.

Amend the paragraph beginning on page 25, line 22 as follows:

Further, an α -chain G-domain peptide is also preferable as a peptide in the region relating to the cell adhesion of LN protein, which is considered to be especially important to the functional expression of epithelial cells, vascular endothelial cells, muscle cells, neural cells (neurons), etc., and for instance, Arg-Lys-Arg-Leu-Gln-Val-Gln-Leu-Ser-Ile-Arg-Thr (AG73) (SEQ ID NO: 1), Leu-Gln-Gln-Arg-Arg-Ser-Val-Leu-Arg-Thr-Lys-Ile (AG73T) (SEQ ID NO: 2), Thr-Leu-Gln-Leu-Gln-Glu-Gly-Arg-Leu-His-Phe-Met (AG76.8) (SEQ ID NO: 17), Thr-Leu-Gln-Leu-Gln-Glu-Gly-Arg-Leu-His-Phe-Nle (AG76.8X) (SEQ ID NO: 18), Val-Lys-Thr-Glu-Tyr-Ile-Lys-Arg-Lys-Ala-Phe-Met (AG81.2) (SEQ ID NO: 3), Val-Lys-Thr-Glu-Tyr-Ile-Lys-

Arg-Lys-Ala-Phe-Nle (AG81.2X) (SEQ ID NO: 4), Lys-Asn-Arg-Leu-Thr-Ile-Glu-Leu-Glu-Val-Arg-Thr (A2G73) (SEQ ID NO: 5), Lys-Pro-Arg-Leu-Gln-Phe-Ser-Leu-Asp-Ile-Gln-Thr (A3G72) (SEQ ID NO: 6), Lys-Phe-Leu-Glu-Gln-Lys-Ala-Pro-Arg-Asp-Ser-His (A4G73) (SEQ ID NO: 19), Gly-Glu-Lys-Ser-Gln-Phe-Ser-Ile-Arg-Leu-Lys-Thr (A4G78) (SEQ ID NO: 20), Thr-Leu-Phe-Leu-Ala-His-Gly-Arg-Leu-Val-Phe-Met (A4G82) (SEQ ID NO: 7), Thr-Leu-Phe-Leu-Ala-His-Gly-Arg-Leu-Val-Phe-Nle (A4G82X) (SEQ ID NO: 8), Gly-Pro-Leu-Pro-Ser-Tyr-Leu-Gln-Phe-Val-Gly-Ile (A5G71) (SEQ ID NO: 9), Arg-Asn-Arg-Leu-His-Leu-Ser-Met-Leu-Val-Arg-Pro (A5G73) (SEQ ID NO: 10), Arg-Asn-Arg-Leu-His-Leu-Ser-Nle-Leu-Val-Arg-Pro (A5G73X) (SEQ ID NO: 11), Leu-Val-Leu-Phe-Leu-Asn-His-Gly-His-Phe-Val-Ala (A5G77) (SEQ ID NO: 12), Leu-Val-Leu-Phe-Leu-Asn-His-Gly-His (A5G77f) (SEQ ID NO: 13), etc., which are derived from mouse LN, and Lys-Asn-Ser-Phe-Met-Ala-Leu-Tyr-Leu-Ser-Lys-Gly (hA3G75) (SEQ ID NO: 21), Gly-Asn-Ser-Thr-Ile-Ser-Ile-Arg-Ala-Pro-Val-Tyr (hA3G83) (SEQ ID NO: 15), etc., which are derived from human LN, are exemplified. The cell adhesion peptides are available through ordinary methods for synthesizing peptides.

Amend the Table 1 beginning on page 48, line 20 as follows:

(Table 1)

Laminin α -chain G-peptides	Amino acid sequences	Inhibition degree of cell adhesion		Binding strength to T2 cells	
		Free peptide	Heparin	Ratio to FIB-1	Comprehensive evaluation
AG73	RKRLQVQLSIRT (SEQ ID NO: 1)	+++	+++	100	AA
AG73T	LQRRSVLRTKI (SEQ ID NO: 2)	No	+		C
AG76.8	TLQLQEGRLHFM (SEQ ID NO: 17)	No	+		C
AG81.2	VKTEYIKRKAFM (SEQ ID NO: 3)	No	++	< 1	C
A2G72-A2G73	KNRLTIELEVRT (SEQ ID NO: 5)	No	++		C
A3G72	KPRLQFSLDIQT (SEQ ID NO: 6)	+++	+++	10	A
A4G78	GEKSQFSIRLKT (SEQ ID NO: 20)	No	+++		C
A4G82	TLFLAHGRLVFM (SEQ ID NO: 7)	+++	+++	10	A
A5G71	GPLPSYLQFVGI (SEQ ID NO: 9)	+++	No	< 1	B
A5G73	RNRLHLSMLVRP (SEQ ID NO: 10)	+	++		C
A5G77	LVLFLNHGHFVA (SEQ ID NO: 12)	+++	+		B
hA3G75	KNSFMALYLSKG (SEQ ID NO: 21)	+++	+	2	B
hA3G83	GNSTISIRAPVY (SEQ ID NO: 15)	+++	+	1-2	B
FIB-1	YAVTGRGDSPAS (SEQ ID NO: 16)	++	No	(1)	B

AA: extremely strongly binding/adhering

A: strongly binding/adhering

B: intermediately binding/adhering (as the same degree as FIB-1 peptide)

C: more weakly binding/adhering than FIB-1 peptide

Amend the paragraph beginning on page 54, line 17 as follows:

50 µl of 2-20 µg/ml MAST-GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro) (SEQ ID NO: 22) and MMAC-GRGDSP (SEQ ID NO: 22) dissolved in 50% ethanol were poured into wells, and air-dried/immobilized. Next, the wells to which MAST-GRGDSP (SEQ ID NO: 22) and MMAC-GRGDSP (SEQ ID NO: 22) (0.1-1.0 µg/well) were coated were rinsed with the serum-free DMEM medium. T2 cells suspended in the medium at the concentration of 6×10^4 cells/100 µl were seeded, and cultured in a CO₂ incubator at 37 °C for 24 hours. Then, the cells were fixed with methanol, stained, and measured its absorbance according to the method in Example 7. The results are shown in FIG. 15. A culture wherein FN was coated as a standard material was conducted simultaneously. The cell adhesion on FN was shown as a standard (100%).

Amend the paragraph beginning on page 54, line 29 as follows:

Ordinary proteins are denatured and inactivated under the condition of 50% ethanol. However, as is shown in Examples 21 and 22, pseudomatrices, not only MAST/MMAC-GRGDSP (SEQ ID NO: 22), exhibited the stable cell adhesion ability even when it was dissolved in 50% ethanol (see Examples 21, 22, and FIGS. 18, 19). This performance was not lost even after the coated wells were left for a long time at room temperature.

Amend the paragraph beginning on page 55, line 6 as follows:

The adhesion of T2 cells to both pseudomatrices is slightly lower than that of the standard cell adhesion protein FN. In a case of GRGDSP (SEQ ID NO: 22) peptide, there is only

one amino acid residue, -CO·NH-Gly- that can serve as a spacer that secures a distance to a hydrophobic binding-adsorptive polymer and reduce steric hindrance. However, in a case of FIB-1 peptide having the five amino acid residues, -CO·NH-Tyr-Ala-Val-Thr-Gly- (SEQ ID NO: 23) that can serve as a spacer and containing GRGDSP (SEQ ID NO: 22) sequence, the same level of cell adhesion ability as that of FN was exhibited (see Examples 21, 22, and FIGS. 18, 19). Therefore, it is considered that at least one of the reasons why the amount of T2 cell adhesion is slightly low in the case of MAST/MMAC-GRGDSP (SEQ ID NO: 22) can be attributable to a steric hindrance to GRGDSP (SEQ ID NO: 22) peptide by hydrophobic binding-adsorptive polymers. The GRGDSP (SEQ ID NO: 22) peptide bound to MAST hydrophobic binding-adsorptive polymer exhibits the larger amount of cell adhesion than that of the same peptide bound to MMAC polymer, because MAST-GRGDSP (SEQ ID NO: 22) exhibits a higher adsorption to polystyrene wells.

Amend the paragraph beginning on page 55, line 25 as follows:

Example 19 (Adhesion of T2 cells to wells wherein MAST-GRGDSP (SEQ ID NO: 22) pseudomatrix dissolved in aqueous solution containing ethanol and air-dried/immobilized, and concentration dependency of competitive inhibition by free GRGDSP (SEQ ID NO: 22) peptide)

Amend the paragraph beginning on page 56, line 29 as follows:

In the same manner as in Example 18, MAST-GRGDSP (SEQ ID NO: 22) was dissolved in 50% ethanol, air-dried/immobilized on wells, and T2 cells were seeded/cultured thereon.

Separately, just before the cells were seeded, 0.25-4.0 mg/ml of free GRGDSP (SEQ ID NO: 22) peptide was added to the cell suspension, and the culture and the subsequent measurement of absorbance were conducted in the same manner as described above. The results are shown in FIG. 16. With the increase in the concentration of free GRGDSP (SEQ ID NO: 22) peptide, the cell adhesion was gradually inhibited and almost completely inhibited at 4.0 mg/ml. This suggests that T2 cells specifically bind/adhere through immobilized GRGDSP (SEQ ID NO: 22). Further, when the applied amount of MAST-GRGDSP (SEQ ID NO: 22) was decreased, the immobilized amount of GRGDSP (SEQ ID NO: 22) decreased, and the competitive inhibition by free GRGDSP (SEQ ID NO: 22) peptide worked more effectively.

Amend the paragraph beginning on page 56, line 14 as follows:

Example 20 (Adhesion of T2 Cells to wells wherein MMAC-GRGDSP (SEQ ID NO: 22) pseudomatrix dissolved in aqueous solution containing ethanol and air-dried/immobilized, and concentration dependency of competitive inhibition by free GRGDSP (SEQ ID NO: 22) peptide)

Amend the paragraph beginning on page 56, line 18 as follows:

In the same manner as in Example 18, MMAC-GRGDSP (SEQ ID NO: 22) was dissolved in 50% ethanol, air-dried/immobilized on wells, and T2 cells were seeded/cultured thereon. Separately, just before the cells were seeded, 0.25-4.0 mg/ml of free GRGDSP (SEQ ID NO: 22) peptide was added to the cell suspension, and the culture and the subsequent measurement of absorbance were conducted in the same manner as described above. The results

are shown in FIG. 17. As with the case with Example 19, with the increase in the concentration of free GRGDSP (SEQ ID NO: 22) peptide, the cell adhesion was gradually inhibited, and almost completely inhibited at 1.0-4.0 mg/ml. This suggests that T2 cells specifically bind/adhere through immobilized GRGDSP (SEQ ID NO: 22) as in Example 19.

Replace the paper sequence listing with the paper sequence listing submitted on March 9, 2011, and enter this into the specification.